OBSERVATIONS ON THE NUTRITION OF MONOGENETIC TREMATODES

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Relatively little information is available regarding the general pattern of nutrition in the Trematoda Monogenea, but there are indications that the two sub-orders of this class of parasitic flatworms differ considerably as regards the nature of their diet. The Monopisthocotylea so far investigated are reported to feed on the epidermal tissues and associated secretions of the host organism, whilst the Polyopisthocotylea appear to be largely sanguinivorous and take in little host tissue other than blood (Goto, 1895; Heath, 1902; Folda, 1928; Gallien, 1934; Sproston, 1945; Llewellyn, 1954; Jennings, 1956, 1959; Uspenskaya, 1962; Kearn, 1963).

Other differences between the two sub-orders, concerned with nutrition, are seen in the cellular structure of the digestive organs. Thus, in the Monopisthocotylea the intestine is lined by a continuous and unpigmented gastrodermis; but in the Polyopisthocotylea the gastrodermis is typically discontinuous and consists of columnar cells, containing varying amounts of brownish or black pigment, interspersed with areas devoid of cells and consisting only of thin basement membrane (Baer and Euzet, 1961). In a number of species the pigment has been identified as hematin, a degradation product of hemoglobin (Llewellyn, 1954; Jennings, 1959).

These differences in gastrodermal structure within the Monogenea are presumably related to the differences in diet and they may reflect, also, further differences in the site and course of the digestive process. In the present investigation, therefore, the relationships between diet, gut structure and digestion in the Monogenea have been studied, as part of a comparative survey of nutrition within this class of Trematoda.

Materials and Methods

The following species of Monogenea, listed systematically with details of their hosts and parasitic locations, have been examined:

MONOPISTHOCOTYLEA

Calicotyle kröyeri Diesing. Cloaca of the thorn-back skate, *Raia clavata* and the starry ray, *Raia radiata*.

Entobdella hippoglossi Müller. Skin and general body surface of the halibut, *Hippoglossus hippoglossus*.

Udonella caligorum Johnston. Egg sacs of copepods (*Caligus* sp.) found on the head and in the buccal cavity of the cod, *Gadus callarias*.

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POLYOPISTHOCOTYLEA

Polystoma integerrimum Fröhlich. Urinary bladder of the common frog, *Rana temporaria.*

Diplozoon paradoxus Nordmann. Gills of the minnow, *Phoxinus phoxinus.*

Discocotyle sagittata Leuckart. Gills of the trout, *Salmo trutta.*

Diclidophora merlangi Kühn. Gills of the whiting, *Gadus merlangus.*

Octodactylus palmata Leuckart. Gills of the ling, *Molva molva.*

Plectanocotyle gurnardi Beneden & Hesse. Gills of the grey gurnard, *Trigla gurnardus.*

To determine the food of each species, and to study the structure of the gut, specimens were fixed in Bouin, Susa, or 10% formalin immediately after removal from the host, and serial sections cut at 5 μ after impregnation and embedding in polyester wax (m.pt. 37° C.) or paraffin wax (m.pt. 56° C.). For identification of intestinal contents sections were examined by one or other of the following methods:

1. The alcian blue method for mucins (Steedman, 1950).
2. The periodic acid-Schiff (P.A.S.) method for mucins and carbohydrates.
3. The mercuric bromphenol blue method for proteins (Mazia, Brewer and Alfert, 1953).
4. The benzidine method for hemoglobin (Pickworth, 1934).
5. The application of various solubility and bleaching tests for hematin (summarized by Jennings, 1959).
6. The Gmelin test for hematoidin and bile pigments.
7. The Turnbull's and Prussian blue methods for ferrous and ferric salts.
8. Various routine histological methods, *e.g.,* hematoxylin and eosin, Mallory's trichrome stain, Feulgen's reaction for nuclei, etc.

To aid identification of the chosen food the host organs were fixed and examined by the above methods, for comparison of tissue components with the trematode's intestinal contents. Further, where the trematodes had obviously only recently fed, they were induced to regurgitate the food, by gentle pressure, and the material so obtained examined either fresh or after treatment as a fixed and stained smear.

In the study of the feeding mechanisms the trematodes were observed alive upon their hosts, whenever this was possible, and others were fixed and sectioned *in situ.* The latter process was facilitated by fixation in warm (40° C.) Bouin, or by plunging the host organ and attached flatworms into isopentane, cooled to − 160° C. in liquid nitrogen, followed by transfer of the frozen mass into fixative held at − 1° C.

The site and course of digestion were investigated by isolating recently fed trematodes in aerated salt or fresh water (Hédon-Fleig saline with added glucose for *Polystoma*) and fixing individuals at progressive intervals up to three days, the maximum survival time for most species. The progressive breakdown and absorption of the food was followed in sections prepared and treated as above. Enzyme activity in the alimentary system was investigated histochemically, using frozen or 45° C. paraffin wax sections prepared after fixation at − 1° C. in 10% formalin buffered to pH 7.0. The histochemical methods employed included the indoxyl acetate method for non-specific esterases (Holt, 1958), both metal-salt and azo-dye methods for alkaline and acid phosphatases (Gomori, 1952; Burstone, 1958), the

Tween 80 method for lipase (Gomori, 1952) and the L-leucyl-β-naphthylamide method for leucine aminopeptidase (Burstone and Folk, 1956).

Observations

MONOPISTHOCOTYLEA

1. *Calicotyle kröyeri*

Calicotyle kröyeri feeds exclusively on epidermal cells and mucoid secretions derived from the lining of the skate cloaca. In many instances the gut lumen of specimens fixed immediately after removal from the host contained mucus, staining strongly with alcian blue and P.A.S., together with numerous large cells 10–12 μ in diameter and containing prominent nuclei (Fig. 1). These cells are identical with the epidermal cells *in situ* on the cloacal wall or lying free in the mucoid material coating the walls of the cloacal chamber.

The mouth in *C. kröyeri* is anterior and ventral, and surrounded by a poorly defined oral sucker. The anterior lip of the sucker contains unicellular glands whose secretions are used for adhesion to the cloacal wall, and the posterior portion bears a tongue-like valve which on contraction cuts off the cavity of the sucker from the rest of the alimentary system (Fig. 2). The pharynx is highly muscular and devoid of gland cells, and is used to suck in the semifluid mucus and desquamated epidermal cells which are always present in the cloaca. The cloacal wall and its epidermis are always intact and undamaged, even when many specimens of *Calicotyle* are present, and it appears that the pharynx never removes living epidermal cells or breaches the epidermis.

The pharynx leads *via* a short esophagus into the intestine, which consists of two simple unbranched ceca. The esophagus is surrounded by many acidophilic gland cells (Fig. 2) which open into its lumen, but the function of their secretion remains unknown.

The intestinal ceca are lined by a single-layered continuous gastrodermis, made up of columnar cells 16–18 μ tall and 6–8 μ wide, with granular cytoplasm and basal vesicular nuclei (Fig. 1). The cells go through a secretory cycle in which a small vacuole appears basally and then increases in size as it moves to the distal portion of the cytoplasm. The vacuoles eventually pass out into the gut lumen where they may remain as visible and discrete structures for varying periods before they finally disappear.

The entire gastrodermis consistently shows a strongly positive reaction for non-specific esterases, apart from the vacuoles whose finely granular contents remain unstained (Fig. 3).

The mucoid and cellular elements of the food are progressively homogenized as they lie in the gut lumen, demonstrating the occurrence of extracellular digestion. The enzymes responsible for this originate, presumably, in the esophageal glands and from the vacuoles released by the gastrodermis. No inclusions were seen in the gastrodermal cells, apart from the vacuoles, but the intense esterase activity seen in the cytoplasm suggests the occurrence of some intracellular digestion following absorption of partially digested material from the gut lumen.

Non-specific esterases are found also in the cuticle, notably that of the anterior ventral region and that lining the oral sucker, and may be used by the trematode

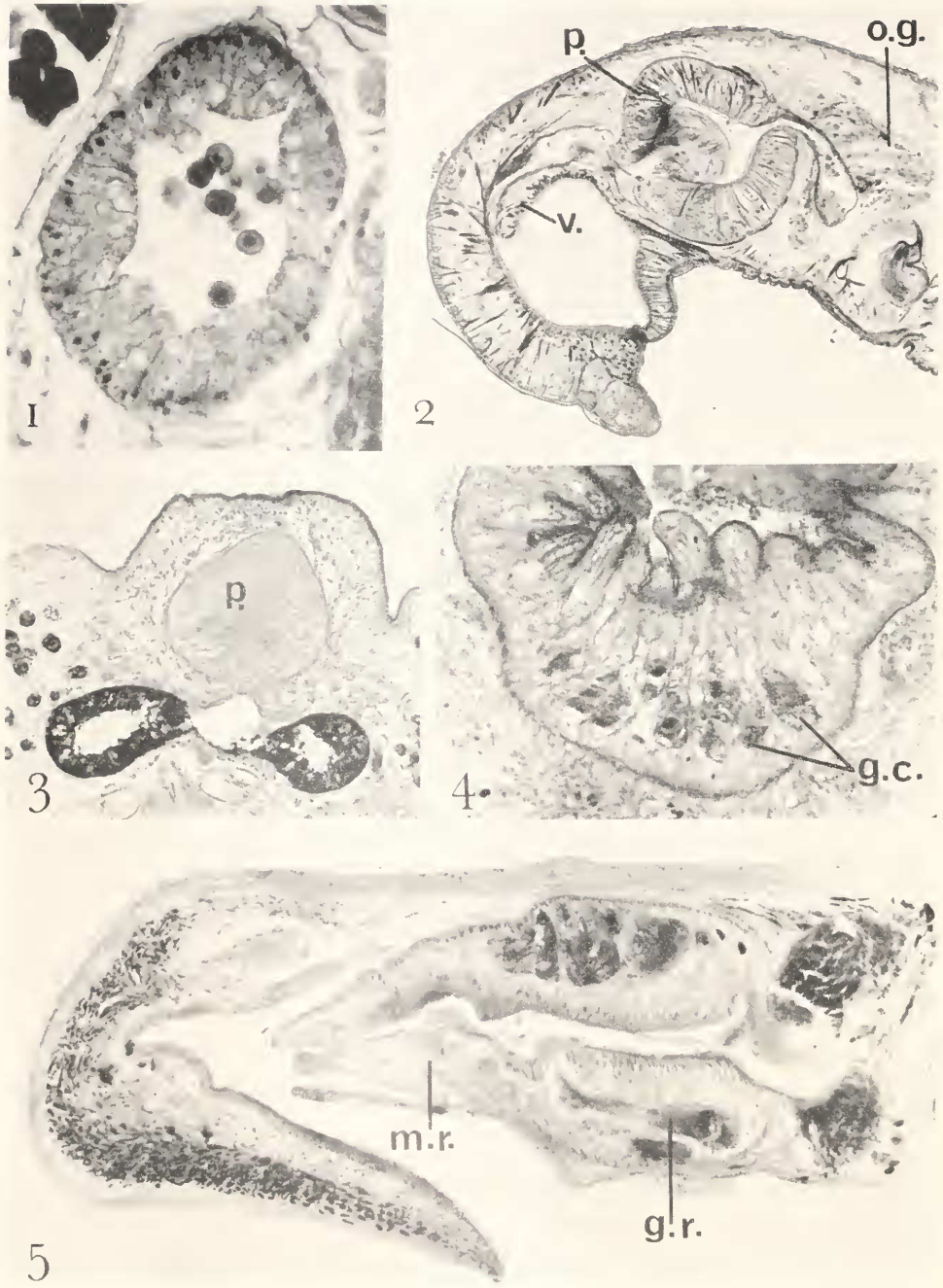

FIGURE. 1. *Calicotyle kröyeri.* Transverse section of an intestinal cecum showing the vacuolated continuous gastrodermis and, in the lumen, recently ingested host epidermal cells. Hematoxylin, eosin and alcian blue. Scale: 1 cm. = 20 μ.

in some form of extracorporeal digestion to accelerate the sloughing-off of spent cells from the cloacal epidermis.

2. Entobdella hippoglossi

Examination of the intestinal contents of *E. hippoglossi* fixed immediately after removal from the host showed that in this species, as in *Calicotyle,* the food consists entirely of host epidermis and mucus, and no traces were found of ingested dermal tissue components such as chromatophores or blood cells.

The ventral subterminal mouth leads directly into the pharynx, which is considerably modified from the usual trematode type to form a muscular-glandular feeding organ (Fig. 5). The anterior portion of this organ is entirely muscular, with flexible lips, and can be protruded through the mouth for application to the host epidermis. The posterior portion is glandular and consists of 40–50 large acidophilic gland cells, separated from each other by muscle fibers. Each cell communicates individually with the lumen of the anterior part of the feeding organ by a fine duct, and each duct opens distally at the apex of a large papilla (Fig. 4).

The gland cells give no reaction with the indoxyl acetate method for non-specific esterases, but fresh frozen sections applied to thin films of solidified 2% aqueous gelatine cause liquefaction and cavitation in the area covered by the glandular portion of the feeding organ, indicating the presence of a proteolytic enzyme. The feeding organ of *E. soleae* is reported to produce a similar gelatine-splitting protease (Kearn, 1963), and it seems likely, therefore, that protease production in the pharynx is characteristic of the entobdellid trematodes as a group.

It was not possible to observe *E. hippoglossi* in the act of feeding, but the presence on the host skin of circular lesions of the approximate diameter of the feeding organ indicates that the proteolytic secretions are used to erode and dissolve epidermal tissue prior to ingestion. This is supported by the fact that relatively few intact epidermal cells are found in the intestinal contents, even when the gut is full and the trematode obviously only recently fed. Generally the gut contents are quite homogeneous, acidophilic and stain only lightly with alcian blue or P.A.S., in marked contrast to the situation seen in *Calicotyle.*

The feeding organ leads posteriorly into the intestine *via* a short esophagus, into which open the ducts of numerous unicellular glands lying in the parenchyma of the anterior portion of the body. These esophageal gland cells are intensely basophilic but the function of their secretion could not be detected.

FIGURE 2. *Calicotyle kröyeri.* Longitudinal section through the anterior region. o. g., oesophageal glands; p., pharynx; v, tongue-like valve which can close off the pharynx from the oral sucker. Mallory. Scale 1 cm. = 200 μ.

FIGURE 3. *Calicotyle kröyeri.* Horizontal longitudinal section of the anterior region, showing the pharynx (p.) and portions of the two intestinal ceca. The gastrodermis in each cecum shows intense non-specific esterase activity. Holt indoxyl acetate method. Scale: 1 cm. = 50 μ.

FIGURE 4. *Entobdella hippoglossi.* Transverse section through the posterior glandular region of the feeding organ, showing the gland cells (g. c.) and papillae. Mallory. Scale: 1 cm. = 75 μ.

FIGURE 5. *Entobdella hippoglossi.* Longitudinal section through the anterior region showing the muscular-glandular feeding organ. g. r., glandular region; m. r., muscular region. Hematoxylin and eosin. Scale: 1 cm. = 125 μ.

The intestine is divided into two ceca which re-unite posteriorly by means of a commissure and give off over their entire length branched diverticula. It is lined throughout by a continuous gastrodermis consisting of uniform flattened cells, 12–15 μ long and 5–7 μ tall, with finely granular cytoplasm and basal vesicular nuclei. Gland cells are absent and no enzyme activity could be demonstrated. The only variation observable in the gastrodermis is in the height of the constituent cells, and this is related to the amount of food present in the lumen, the cells becoming even more flattened as the intestinal walls stretch to accommodate newly ingested material.

The amount of material in the gut lumen decreases with time, after feeding, but without noticeable change in consistency from the relatively homogeneous condition in which the food is ingested. This fact, together with the absence of gland cells from the gastrodermis, suggests that the bulk of digestion in *E. hippoglossi* is effected by the secretions poured on to the food from the glands of the feeding organ before and during ingestion, aided perhaps by the secretions of the esophageal glands. The gastrodermis would thus appear to be entirely absorptive in function and to play little or no part in the production of the digestive juices.

3. *Udonella caligorum*

Udonella caligorum lives attached to the egg sacs of copepods (*Caligus* sp.) which in turn are ectoparasitic in the buccal cavity and on the head region of cod, halibut and ling.

The only recognizable material found amongst the gut contents of *Udonella* was a mucoid substance staining lightly with alcian blue and P.A.S., and often the intestine contained only a finely granular acidophilic digest. Nothing can be seen to suggest that *Udonella* feeds on the copepod tissues or body fluids, and it is concluded that the trematode ingests mucus, and perhaps sloughed-off epidermal cells, from the fish skin or mucous membrane adjacent to the copepod's point of attachment.

The mouth in *Udonella* is anterior and ventral, and leads directly into the large muscular pharynx. This can be protruded slightly through the mouth but is not armed or equipped with glandular elements so that it is unlikely that it penetrates host tissues. Feeding, therefore, is probably a case of merely sucking in the material lying on the fish epidermis.

The intestine in *Udonella,* in contrast to that in most other Monopisthocotylea, is undivided and extends almost to the posterior end of the body as a simple sac, reminiscent of the sac-like gut of many rhabdocoel Turbellaria. It is lined by a flattened and continuous gastrodermis similar to that found in *Entobdella.*

Digestion appears to be entirely intraluminar, judging from the appearance of the gut contents, and nothing was seen to indicate intracellular digestion, as the gastrodermis shows no trace of esterase activity.

POLYOPISTHOCOTYLEA

1. *Polystoma integerrimum*

Polystoma integerrimum is sanguinivorous, feeding on blood drawn from the capillaries of the frog urinary bladder, and no host tissues other than blood were found in the gut contents.

The ventral mouth is encircled by an oral sphincter and leads into the cavity of the oral sucker. This is lined by cuticle and surrounded by numerous unicellular glands which open *via* long branched ducts over the external surface of the sucker and also into the oral cavity. The glands produce a granular proteinaceous secretion which stains strongly with the Mallory and Mazia methods, but gives no reaction for esterases or phosphatases. The distribution of the ducts conveying the secretion to the exterior indicates that it is probably used in adhesion.

The oral cavity is linked with the large muscular and bulbous pharynx by means of a short cuticle-lined buccal tube, into which the anterior portion of the pharynx projects. The wall of the pharynx contains, in addition to muscular elements, a number of large cells with prominent nuclei and nucleoli, and a series of small vacuoles of unknown function ranged along the inner and outer surfaces at regular intervals (Fig. 6).

The pharynx leads *via* the esophagus into a bifid intestine whose ceca run the length of the body and give off branches which in turn repeatedly subdivide and anastomose. The esophagus is a short muscular tube surrounded by numerous unicellular glands arranged in two distinct zones. The cells of the inner zone, immediately around the esophagus, are smaller and produce a granular secretion giving an intensely positive reaction for alkaline phosphatase, while the outer larger cells produce a more coarsely granular and strongly acidophilic secretion quite free of phosphatases (Fig. 7). Fresh frozen sections and aqueous extracts of the esophageal region rapidly cause cavitation in gelatine films, indicating the production of a proteolytic enzyme by these esophageal glands, but it was impossible to determine which type of gland cell was responsible.

Both types of gland cell discharge through long unbranched ducts which enter the pharynx at its posterior end and run forward between the cuticular lining and the underlying musculature to open finally into the anterior end of the pharynx lumen (Figs. 6 and 7).

During feeding the oral sucker is flattened and flared against the bladder wall, and contraction of radial muscles within the sucker draws up a plug of bladder tissue whose tip reaches the anterior end of the pharynx. The plug is held secure by the constricting grip of the oral sphincter around its base while the sucking action of the pharynx, aided no doubt by proteolytic secretions from the esophageal glands, ruptures capillaries and draws blood into the intestine. Bladder tissue is never ingested, however, and very little damage is caused to the bladder epithelium.

The structure of the gastrodermis in *Polystoma* has been described elsewhere (Jennings, 1959). In brief, the gastrodermis is a single-layered discontinuous structure made up of columnar cells 16–18 μ tall and 8–10 μ wide, with basal vesicular nuclei and cytoplasm containing varying amounts of the pigment hematin, interspersed with areas devoid of cells and where only a thin basement membrane separates the gut lumen from underlying body tissues (Fig. 8). The hematin is contained within spherical vesicles up to 8 μ in diameter and the number of these increases with age, so that a mature cell is loaded with pigment and the nucleus obscured. When this condition is reached the vesicles are extruded into the gut lumen or, more commonly, the entire cell disintegrates either *in situ* or after being shed from the gastrodermis. The vesicles themselves persist intact for some time, but eventually rupture to discharge their contained hematin. New, younger cells

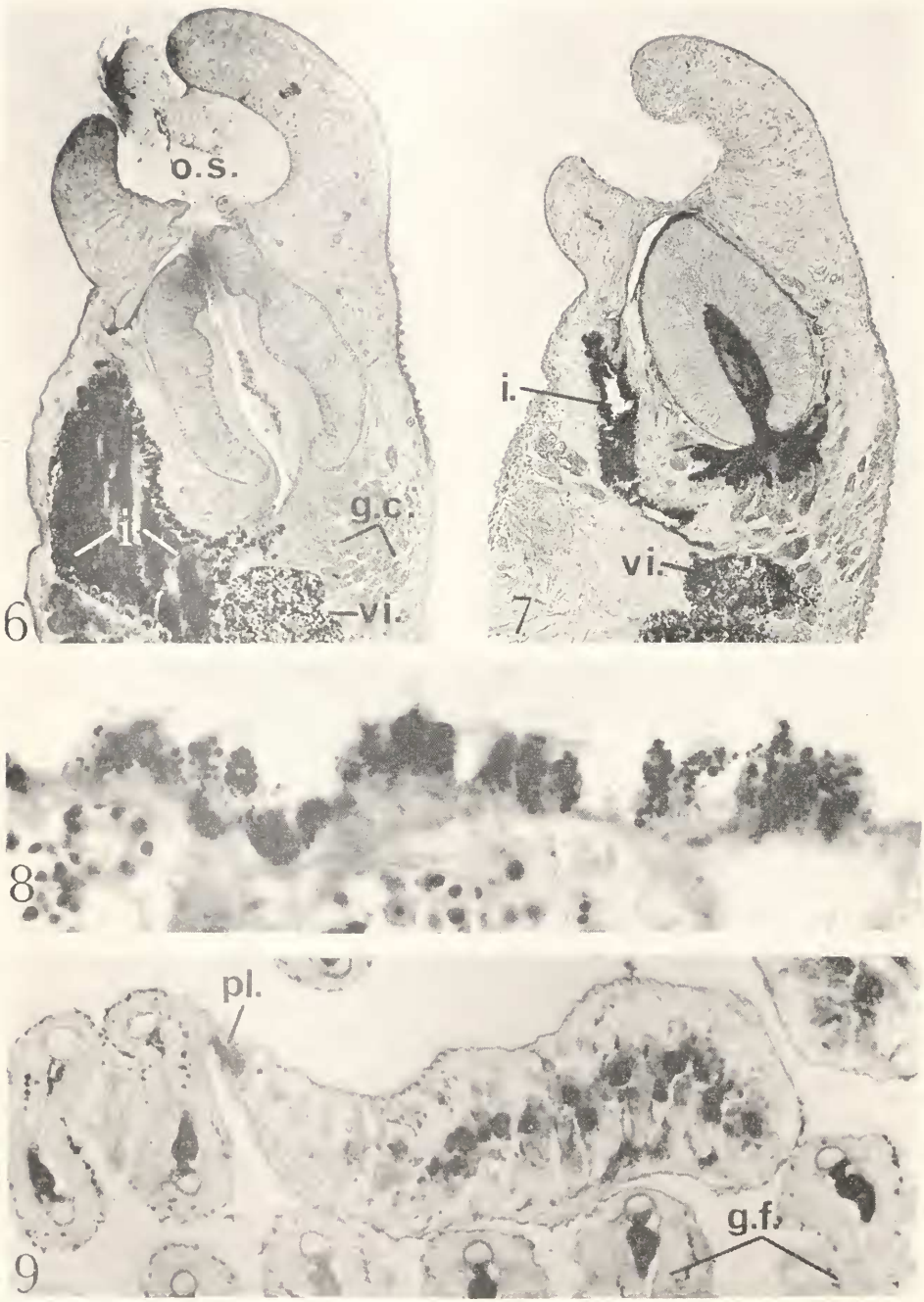

FIGURE 6. *Polystoma integerrimum.* Longitudinal section of the anterior region. g. c., gland cells posterior to the pharynx whose ducts run forward between the inner cuticular lining and musculature of the pharynx and open in its anterior portion; i., intestine, lined by a dis-

grow up to replace the spent cells and fill in the gaps in the gastrodermis, and thus the latter structure is in a state of constant degeneration and renewal.

Digestion in *Polystoma* occurs by a combination of extracellular and intracellular processes. Erythrocytes entering the intestine are immediately hemolyzed and within three hours of ingestion their nuclei have also disintegrated. The freed nuclear material mixes with the other gut contents and causes the whole mass to stain lightly with Feulgen, but this reaction eventually disappears as digestion progresses.

The intraluminar phase of digestion is accompanied by absorption of semi-digested substances by the smaller younger cells of the gastrodermis, and their cytoplasm becomes swollen with spherical aggregations of material showing the same staining reactions as that remaining in the gut lumen. These cells show intense alkaline phosphatase activity along their distal margins and this is obviously concerned with the process of absorption. The enzyme is best visualized by the azo-dye method since the black cobalt-sulphide end product of the calcium-salt technique may be masked by any hematin present.

Absorption from the gut lumen continues until no stainable material remains. This situation is reached 24–48 hours after a meal, depending upon the amount of blood ingested. Digestion is completed intracellularly in the vesicles within which material is aggregated as it is absorbed from the lumen, but of the enzymes concerned in the process, only non-specific esterases could be demonstrated histochemically. These are localized within the vesicles and cannot be demonstrated in the cytoplasm of the gastrodermal cells.

As intracellular digestion proceeds, stainable material disappears from the vesicles and is replaced by granules of hematin resulting from degradation of the hemoglobin content of the meal. The hematin remains within the cell and thus the amount seen in a mature cell about to be shed from the gastrodermis probably represents an accumulation from the digestion of several meals.

Extrusion of hematin vesicles or the shedding of intact spent cells occurs 24–48 hours after a meal and consequently there is at this time a marked increase in the amount of hematin lying free in the gut lumen. Many of the freed vesicles, prior to rupturing, still show traces of esterase activity and this confirms a suggestion made in an earlier account (Jennings, 1959) that enzymes concerned primarily with intracellular digestion may remain in the hematin vesicles and be eventually transported to the gut lumen where they are released when the vesicles rupture. Due to the ramifications of the gut in *Polystoma* there is never complete evacuation between meals, and it is likely that these enzymes of intracellular origin will still be

continuous pigmented gastrodermis and containing hematin granules mixed with heavily staining hemolyzed erythrocytes; o. s., oral sucker containing material regurgitated from the intestine; vi., vitellaria. Mallory. Scale: 1 cm. = 75 μ.

FIGURE 7. *Polystoma integerrimum.* Longitudinal section of the anterior region, showing intense alkaline phosphatase activity in the inner zone of gland cells associated with the pharynx. Abbreviations as in Figure 6. Gomori azo-dye method. Scale: 1 cm. = 75 μ.

FIGURE 8. *Polystoma integerrimum.* Transverse section through the gastrodermis, showing the discontinuous structure and the intracellular aggregations of hematin. Mallory. Scale: 1 cm. = 20 μ.

FIGURE 9. *Diplozoon paradoxum.* Longitudinal section of an individual fixed *in situ* on the host gill. g. f., gill filament; pl., plug of gill tissue drawn up and held by the buccal sucker. P.A.S. Scale: 1 cm. = 250 μ.

present in the lumen when the next meal is taken, and thus contribute to the intraluminar digestive phase.

No specific source of the intraluminar digestive enzymes was located, other than the hematin vesicles, and it seems likely, therefore, that the proteolytic secretions of the esophageal glands will play an important part in extracellular digestion, entering the intestine with the food and initiating hemolysis and nuclear breakdown.

The principal endproduct of hemoglobin digestion in *Polystoma* is hematin but a small proportion of the hemoglobin is converted to hematoidin, an iron-free, acid-soluble crystalline substance closely related to the bile pigments. Hematoidin crystals are only rarely found in histological preparations of *Polystoma,* however, due to their solubility in the standard fixatives, but can be seen in fresh squash preparations of the gastrodermis in about 10% of the cells.

2. *Diplozoon paradoxum*

Diplozoon paradoxum feeds predominantly on blood, but small amounts of gill tissue, epithelial cells and mucus are also found amongst the gut contents.

The adult *Diplozoon* consists of two individuals united in permanent copulation, with organic fusion of their bodies midway along the long axis, so that the composite individual is X-shaped. Each individual retains a terminal ventral mouth opening into a buccal cavity which bears laterally a pair of buccal suckers. An oval, muscular pharynx, devoid of glandular elements, protrudes slightly into the buccal cavity and leads backwards into the intestine. This extends posteriorly in each individual as a single much-branched cecum, and where the bodies of the two individuals fuse, the two ceca unite by a median canal, so that the two intestines are confluent.

Diplozoon lives attached to the gills of the host minnow by the clamps of the two opisthaptors and during feeding one or both of the anterior ends attaches itself to a gill filament by means of the buccal suckers. The grip is aided by adhesive secretions produced by clusters of gland cells around the buccal cavity which open on to the anterior body surface. The buccal suckers draw up a plug of gill tissue (Fig. 9), in much the same manner as the oral sucker in *Polystoma* draws up a plug of bladder tissue. The plug extends through the buccal cavity to the pharynx, which is protruded slightly and applied to the tip. Prolonged suction bursts the superficial blood capillaries, and blood, together with a small amount of gill tissue, enters the intestine. There is no evidence indicating the use of histolytic secretions to effect rupture of the gill capillaries, and no serious damage is caused to the gill filaments by the feeding activities of the trematode.

The gastrodermis resembles that of *Polystoma* in that it is a discontinuous and deciduous structure whose individual cells contain the characteristic hematin-laden vesicles. The cells are interspersed with areas of basement membrane either devoid of cells or covered by thin, extremely flattened and unpigmented young cells.

The course of digestion follows closely that observed in *Polystoma,* hemolysis of the erythrocytes occurring during or very soon after ingestion and being followed by partial intraluminar digestion. Soluble substances are absorbed by the gastrodermis and digestion subsequently completed intracellularly, with the production of hematin as a visible insoluble endproduct. As in *Polystoma* the cells actively absorbing materials from the gut lumen show intense alkaline phosphatase activity

distally and this decreases as the cell ages and reduces its digestive functions. No esterase reaction could be demonstrated in the gastrodermis, but the entire nervous system shows intense cholinesterase activity and this provides a simple but effective means of demonstrating the system *in toto* (Halton and Jennings, 1964).

The vitelline glands of the reproductive system are in intimate contact with the intestine for most of its length and show at all times positive reactions for alkaline phosphatase, lipase and aminopeptidase, indicating metabolic activity possibly concerned with absorption and utilization of food materials from the gastrodermis.

In a few instances the intestine of newly fed *Diplozoon* contained, in addition to the hemolyzed blood, a number of reddish needle-shaped crystals 150–200 μ in length. These were water-soluble but could be fixed in absolute ethyl or methyl alcohol, when they stained strongly with the benzidine technique for hemoglobin. The crystals gradually disappeared in the living animal as digestion progressed, and they probably resulted from crystallization of hemoglobin released from hemolyzed erythrocytes and concentrated by absorption of water from the gut contents during the early stages of digestion.

3. *Discocotyle sagittata*

Discocotyle sagittata appears to feed exclusively on blood drawn from the superficial capillaries of the trout gills.

The mouth is anterior and ventral, and opens into a buccal cavity possessing laterally a pair of very large bilobed buccal suckers. The buccal cavity opens posteriorly into a small muscular non-glandular pharynx.

It was not possible to observe *Discocotyle* in the act of feeding, but judging from the similarities in structure and habit it is likely that the breaching of the host capillaries and the ingestion of blood are effected in the same manner as in *Diplozoon*. The buccal suckers are larger and more powerful than in the latter species, however, while the pharynx is relatively smaller, so that the suckers probably play a greater part in creating the necessary suction. No evidence of the production or use of proteolytic secretions could be found, and no significant amount of damage is caused to the gill filaments by the feeding activities of the trematode.

Neither gill tissues nor mucus were observed in the gut contents of the specimens examined.

The pharynx opens directly into the bifid intestine whose ceca extend to the posterior end of the body and give off numerous lateral branches which in turn subdivide and ramify between the vitellaria and other organs.

The gastrodermis resembles that of *Polystoma* and *Diplozoon,* and is made up of large hematin-laden cells, 18–20 μ long and 4–6 μ tall, which are interspersed with smaller, flattened non-pigmented cells and areas completely devoid of cellular elements.

The appearance of the gastrodermis indicated that digestion in *Discocotyle* follows much the same course as in *Polystoma* and *Diplozoon,* and this was confirmed from histological examination of individuals fixed at progressive intervals after removal from the host. Hemolysis and intraluminar digestion are accompanied by active absorption of the products by the gastrodermis, with subsequent completion of digestion and production of hematin within intracellular vesicles. Absorption is particularly noticeable in the smaller non-pigmented cells, and both these and the larger cells show intense distal alkaline phosphatase activity.

Hematin is eliminated from the gastrodermis by extrusion of the vesicles or by the sloughing off of intact spent cells.

It was not possible to demonstrate the presence of proteolytic enzymes in the gastrodermis, by histochemical methods, but as in *Diplozoon* the vitellaria give strong positive reactions for lipase and aminopeptidase.

4. *Diclidophora merlangi*

Diclidophora merlangi feeds chiefly upon blood but small amounts of gill tissue and mucus are also ingested.

The mouth is ventral and subterminal, and opens into a typical buccal cavity with lateral paired buccal suckers. The pharynx is spherical, muscular and devoid of glandular elements, and feeding is effected by suction of the host tissue.

A long esophagus links the pharynx with the bifid intestine whose ceca give off lateral much-branched diverticula. The latter are enveloped by the numerous vitellaria of the reproductive system.

The gastrodermis in *Diclidophora*, as in the other Polyopisthocotylea already described, is a discontinuous and deciduous structure whose cells contain varying amounts of hematin and show the characteristic distal zone of alkaline phosphatase activity. The cells are much smaller than in the other genera investigated, however, and even when fully mature and loaded with hematin are only 6–8 μ long and 3–4 μ tall.

Digestion in *Diclidophora* is effected by a combination of extra- and intracellular processes and follows much the same course as in *Polystoma*, except that hematin appears to be the sole endproduct of hemoglobin degradation and no traces of hematoidin were found. A small amount of non-specific esterase activity can usually be demonstrated in the gut contents of specimens fixed soon after feeding, but this does not increase in amount with time and appears, in fact, to be derived from the gill tissue ingested along with mucus as the subsidiary component of the diet. In control sections of whiting gill approximately 10% of the epithelial cells showed non-specific esterase activity and it is likely that the activity seen in the *Diclidophora* gut contents originates in these cells.

The gastrodermal cells show no enzyme activity, other than alkaline phosphatase, that could be detected by the techniques used, but the vitellaria, as in the other genera studied, give positive reactions for lipase and aminopeptidase.

5. *Octodactylus palmata*

Octodactylus palmata feeds predominantly on blood drawn from the host gill capillaries but as in *Diclidophora* and *Diplozoon*, this diet is supplemented by gill tissue and mucus.

The terminal mouth opens into a buccal cavity which possesses a pair of large lateral buccal suckers. Gill tissue is drawn up through the mouth, and suction by the bulbous and highly muscular pharynx ruptures the capillaries and draws blood into the intestine. The pharynx is devoid of gland cells and its action in procuring the food appears to be entirely mechanical.

The gut contents of recently fed *Octodactylus* generally include mucus and gill tissue in somewhat larger quantities than are found in *Diclidophora* and *Diplozoon*, but no appreciable damage to the gill filaments of the host was observed.

The intestine is of the usual polyopisthocotylean type, being bifid with the ceca of considerable length and giving off many branched lateral diverticula.

The gastrodermis differs somewhat from that of the other genera examined in that only relatively few areas are completely devoid of cells at any one time, and these are usually restricted to the walls of the two main ceca. The cells are small, as in *Diclidophora,* and range from 3–8 μ in height and 6–8 μ in width. The great majority of the cells contain hematin but the pigment granules are generally all confined within a single large vesicle, 3–6 μ in diameter, rather than distributed amongst four or five smaller vesicles as, for example, in *Polystoma.* The larger-sized vesicles often fill the entire cell and displace the nucleus to one side away from its normal basal position. In fixed preparations the vesicles appear as solid masses of hematin, but in fresh squashes the individual pigment granules are free and exhibit constant Brownian movement within the confines of the vesicle.

Digestion in *Octodactylus* follows the pattern observed in the other polyopisthocotyleans studied. Hemolysis is completed very soon after ingestion and then intraluminar digestion is accompanied by absorption and the completion of digestion intracellularly. The gastrodermal cells show a distal zone of high alkaline phosphatase activity which is particularly intensified during absorption.

The hematin resulting from intracellular degradation of hemoglobin accumulates within the vesicles until eventually the distal margins of the individual cells break down and the hematin is discharged into the gut lumen. During this process, and while the cell is recovering, the cell becomes crescent- or cup-shaped and the disorganized distal margin shows only diffuse alkaline phosphatase activity. Cells in this condition may continue to absorb material from the lumen, however, and often show a single small secondary hematin vesicle basally. This increases in size as the cell recovers from expulsion of the primary vesicle and moves distally, almost fills the cell, and is eventually expelled.

The gut contents in *Octodactylus* often show non-specific esterase activity but, as in *Diclidophora,* there is every indication that this originates in the gill tissue and not in the gastrodermis. No other enzymatic activity could be demonstrated histochemically in the intestine, but again lipase and aminopeptidase were abundant in the vitellaria.

6. *Plectanocotyle gurnardi*

None of the specimens of *Plectanocotyle gurnardi* available for examination was recently fed, but since the gastrodermal cells contain at all times large amounts of hematin it is concluded that blood forms the dominant, if not the sole, component of the diet. No traces of gill tissue or mucus were found, but this could conceivably be due to the progress of digestion since the previous meal.

The alimentary system resembles that of *Diclidophora* or *Diplozoon,* with paired buccal suckers, a muscular pharynx and a bifid, much-branched intestine. The gastrodermis is of the typical discontinuous and deciduous type, with somewhat sickle-shaped pigmented cells interspersed with naked areas devoid of cells.

Discussion

These observations on the nutrition of a number of monogenetic trematodes confirm indications available from previous accounts that there is a fundamental difference between the Monopisthocotylea and Polyopisthocotylea as regards the domi-

nant components of the diet. The three monopisthocotyleans studied, from quite different parasitic locations, all feed on the host's epidermis and epidermal secretions, and similar feeding habits have been described in *Entobdella squamata* (Heath, 1902), *Megalocotyle marginata* (Folda, 1928), *Leptocotyle minor* and *Acanthocotyle* sp. (Llewellyn, 1954), *Entobdella solcae, Capsala martinieri, Trochopus* sp. and *Acanthocotyle* sp. (Kearn, 1963). Thus, this type of diet would appear to be a characteristic feature of the Monopisthocotylea. Uspenskaya (1962), however, states that in four other species (*Dactylogyrus vastator, D. solidus, Anchylodiscoides parasiluri* and *Tetraonchus monenteron*) varying amounts of blood are found in the intestinal contents, together with gill tissue and mucus, but the latter substances predominate.

In the Polyopisthocotylea, in marked contrast, blood forms the major, and sometimes the only, component of the diet. Of the species examined in the present study, *Polystoma integerrimum* and possibly *Discocotyle sagittata* feed entirely upon the host's blood, while *Diplozoon paradoxum, Diclidophora merlangi* and *Octodactylus palmata* supplement the blood diet with varying quantities of gill tissue and mucus. Ingestion of blood, or the presence of an intestinal pigment which is presumably hematin and hence indicative of a blood diet, has also been reported in *Hexacotyle* sp., *Onchocotyle* sp., *Octocotyle* sp. and *Microcotyle* sp. (Goto, 1895); *Axine* spp., and *Diclidophora* spp. (Goto, 1895; Llewellyn, 1954); the larval and neotenic adult stages, as well as the normal adult stage, of *Polystoma integerrimum* (Gallien, 1934; Llewellyn, 1954); *Kuhnia scombri* (Sproston, 1945; Llewellyn, 1954); *Hexabothrium appendiculata* and *Anthocotyle merlucci* (Llewellyn, 1954) and *Pricea cybium* and *Protomicrocotyle caranx* (Uspenskaya, 1962).

It is reasonable to suppose that the earliest Monogenea lived ectocommensally upon fish in much the same sort of way as modern Tennocephalida live on crustacean and other hosts. The fish epidermis and its mucoid secretions would form a readily available and rapidly replenished source of food to the flatworm, once the association was established, and by utilizing this the primitive Monogenea would become truly ectoparasitic. On this view the modern monopisthocotylean Monogenea, living as a rule upon the external surface of the host, retain ancestral feeding habits and the only modification found is the evolution in groups such as the entobdellid species of a specific feeding mechanism involving the use of histolytic "salivary" secretions. Even species such as *Calicotyle* which have sought the shelter of the host's cloaca, and are apparently on the way to becoming endoparasitic, still use the original type of food.

The polyopisthocotylean Monogenea, on the other hand, are predominantly gill parasites, having migrated into the branchial chamber of their piscine hosts, and they have departed considerably from the supposedly primitive feeding habits. The highly vascularized gill filaments offer an extremely nutritious and, again, readily available food in the form of blood, and the basic monogenean feeding mechanism of a suctorial pharynx is capable of obtaining this food without any modification other than slight elaboration of the oral and buccal sucker system. Thus, the differences in diet between the Monopisthocotylea and Polyopisthocotylea have not affected the feeding mechanism, and the anterior region of the alimentary system remains remarkably constant in structure throughout the Monogenea and, indeed, the Digenea. This uniformity contrasts sharply with the situation in the Turbel-

laria, where considerable diversification in the form of the pharynx is linked with the utilization of a wide variety of prey, ranging from Protozoa and many other invertebrates to tunicates (Jennings, 1957).

The differences in diet within the Monogenea do, however, have considerable effect upon the cellular structure of the gastrodermis. In the Monopisthocotylea digestion of the food creates no particular problem as regards the elimination of unwanted endproducts, even though the process is completed intracellularly. In the Polyopisthocotylea, however, the diet of blood and the retention of the intracellular digestive phase result in the intracellular production of hematin. The elimination of this insoluble substance is achieved at the expense of the continuity of the intestinal lining, and produces the discontinuous or *deciduous* gastrodermis characteristic of the sub-order. This involves wastage of cellular materials and thus the Polyopisthocotylea appear to be incompletely adapted to a blood diet. A more complete adaptation would be the extracellular formation of hematin, or the degradation of hemoglobin along some other pathway which allows the unwanted iron to be eliminated in a soluble form. The Trematoda are in fact capable of evolving such digestive processes, and these are seen in certain sanguinivorous Digenea such as *Haematoloechus* and *Haplometra* (Halton, unpublished work).

A compensating factor arising from the disintegration of gastrodermal cells in the Polyopisthocotylea is that intracellular enzymes are released to mingle with the gut contents and initiate breakdown of the next meal. Unfortunately it has proved impossible to localize the source of other digestive enzymes in either the Monopisthocotylea or the Polyopisthocotylea with the techniques at present available. It seems likely that the secretions of esophageal glands, poured on to the food during ingestion, play an important part in the extracellular phase of digestion, but this cannot be conclusively demonstrated. Certainly, however, the gastrodermis in the Monogenea has not evolved to the point of specialization of cellular function, for it shows no signs whatsoever of differentiation into glandular and absorptive or phagocytic components. In this respect it differs radically from the gastrodermis of other members of the phylum Platyhelminthes, such as the triclad Turbellaria, and of other acoelomates, such as the Rhynchocoela, where well differentiated gland cells occur and where there is separation of secretory and absorptive or phagocytic functions (Jennings, 1962a; 1962b).

Summary

1. A comparative study has been made of the food, feeding mechanism, gut structure and digestive processes in representatives of the two sub-orders of the Trematoda Monogenea.

2. The two sub-orders differ fundamentally as regards the dominant components of the diet, the Monopisthocotylea feeding on the epidermis and associated mucoid secretions of the host while the Polyopisthocotylea feed primarily upon the host's blood. In some instances the Polyopisthocotylea supplement the diet with small amounts of host tissue and mucus.

3. The feeding mechanism in both groups consists basically of a muscular pharynx, and ingestion is the result of muscular suction, aided in some cases by histolytic secretions produced in pharyngeal or esophageal glands and used to erode the host tissues.

4. The two sub-orders differ considerably with regard to the structure of the gastrodermis, that of the Monogenea being a continuous cellular structure as in most other animals while in the Polyopisthocotylea it is a discontinuous and deciduous structure whose cells contain varying amounts of the pigment hematin.

5. Digestion in both the Monopisthocotylea and Polyopisthocotylea is effected by a combination of extra- and intracellular processes, but in the Polyopisthocotylea intracellular degradation of hemoglobin results in the accumulation within the gastrodermal cells of insoluble hematin, and the elimination of this substance results in the deciduous gastrodermis characteristic of the sub-order.

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